AMBIENT ENDOTOXIN: MEASUREMENT OF CONCENTRATION AND ASSESSMENT OF HEALTH RISK OVER SEMIARID REGION OF INDO- GANGETIC BASIN

A

Synopsis

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Submitted by

Krishma Yadav

Under the supervision of

Dr. Ranjit Kumar
Co-Supervisor
Department of Chemistry
Faculty of Science

Dr. Ashok Yadav
Supervisor
Department of Mechanical
Faculty of Engineering

DEPARTMENT OF CIVIL ENGINEERING, FACULTY OF ENGINEERING

DAYALBAGH EDUCATIONAL INSTITUTE (DEEMED TO BE UNIVERSITY)

DAYALBAGH, AGRA-282005

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1. INTRODUCTION

Endotoxin has been identified as a biomarker, which is a non-allergenic component of bioaerosols. Endotoxin can form alliances with dust particles, resulting in symptoms such as fever, shivering, arthralgia, influenza-like symptoms (malaise), blood leukocytosis, neutrophilic airway inflammation, asthma symptoms such as dry cough, dyspnea, and chest tightness, bronchial obstruction, dose-dependent lung function impairment, and decreased lung diffusion capacity. Understanding endotoxin concentrations in ambient air, seasonal change, climate effects, and sources is vital, and it has become critical in the present pandemic as it is one of the important constituents of bioaerosol.

Many of the most hazardous compounds are of biological origin (toxins), such as botulinum toxin, acetaminophen (Tylenol), endotoxin, and so on. Endotoxin, also known as lipopolysaccharide (LPS), is a poisonous chemical of biological origin with high proinflammatory properties. Richard Friedrich Johannes Pfeiffer discovered LPS and named endotoxin after distinguishing it between exotoxins, which are toxins released by bacteria into the surrounding environment, and endotoxin, which are toxins kept "within" the bacterial cell and released only after the destruction of the bacterial cell wall. LPS is an amphiphilic, heat- stable and water-soluble macromolecule produced by Gram-negative bacteria that govern the biological properties of bacterial endotoxin (Duquenne et al., 2013). Gram-negative bacteria's cell wall antigens (O antigen) are components of LPS.

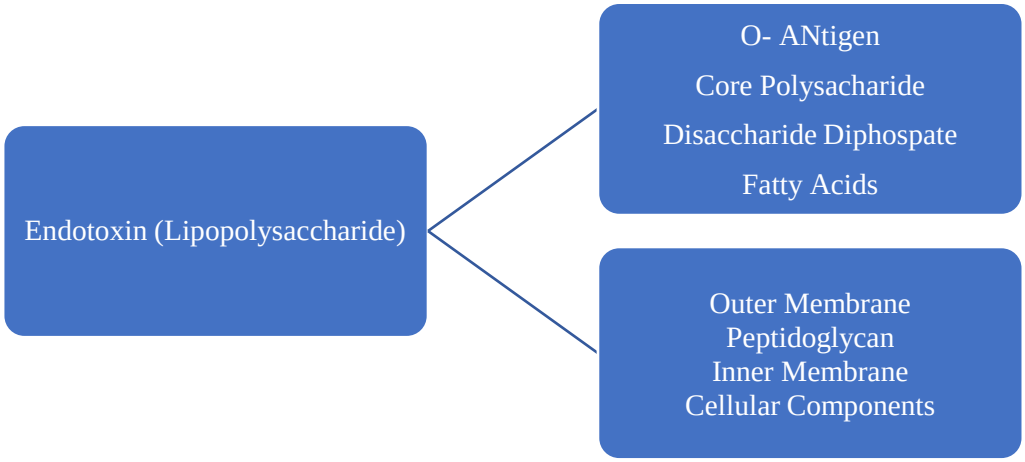

Figure 1. Basic components of endotoxin.

LPS is a big molecule made up of a lipid and a polysaccharide made up of O- antigen, outer core, and inner core that are connected by a covalent bond. Toxicity is linked to the lipid component (Lipid A), while immunogenicity is associated to the polysaccharide components.

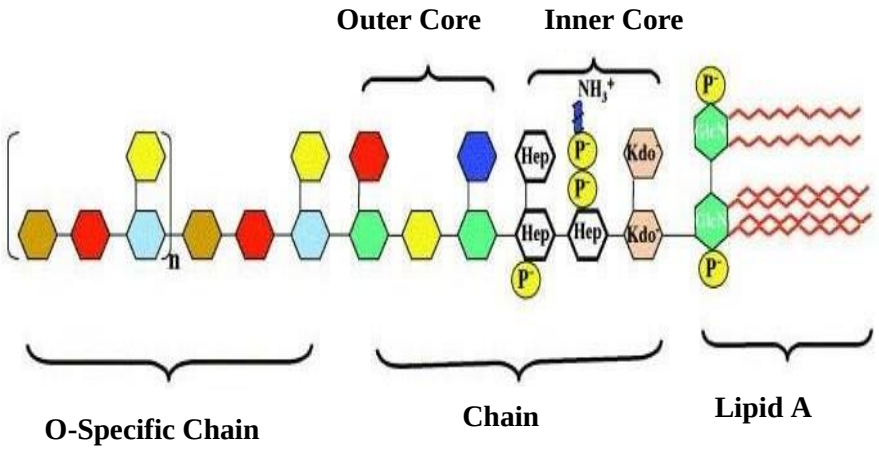

Figure 2. Structure of the cell envelope of a Gram-negative bacterium (Source: Google)

The impact of chemical and biological compounds of natural and manmade origin on the environment, living body, and human health has gotten a lot of attention in recent decades. Chemicals' negative effects on biological systems are a major source of concern. Endotoxin is a naturally occurring biological toxin generated by some bacterial cells. Endotoxin from Gram- negative organisms is prevalent in the environment, the flora of the skin, mouth cavity, and lower intestinal tract, and has gained attention (Klaasen et al., 2001).

Endotoxin contamination in the environment

Endotoxin is a biological component of particulate matter that has been related to negative health consequences. Endotoxin inhalation has been shown to cause chest tightness, a rise in body temperature, and inflammation markers such as blood C- reactive protein; neutrophilic sequestration in the lung and increases in sputum monocytes, neutrophils, and lymphocytes; a decrease in pulmonary function; and an increase in bronchial reactivity in healthy patients.

The atmosphere is made up of a complex of suspended solid and liquid particles with uniform size distributions. Soil dust sea salt, pollutant gas salts, soil fragments, water droplets, combustion soot, and Bioaerosols make up this mixture. Bioaerosol contains endotoxin, which is a biogenic chemical. Biogenic aerosols are biological materials such as microorganism cells/spores, pollen, and plant

fragments that are emitted as aerosols. In nature, bioaerosols can be found all over. As raindrops fall, they may produce bioaerosol.

Bacteria, fungi, viruses, and pollen make up the majority of bioaerosols. The planetary boundary layer (PBL) has the highest bioaerosol concentrations, which decrease with altitude. Climate, ultraviolet (UV) light, temperature and humidity, and resources found in dust or clouds are all biotic and abiotic factors that influence the occurrence of bioaerosols (Acosta-Martnez et al., 2015). Bacteria predominate in bioaerosols created in coastal habitats, while bacteria, fungi, and pollen predominate in bioaerosols formed over land. Depending on the time and region, the abundance of certain bacteria and their food sources vary (Smets et al., 2016)

Endotoxin is similar to a bioaerosol component. Endotoxin is very resistant to desiccation, autoclaving, and distillation after it has been released into the environment (Ludwig and Avis, 1988). It can be found in a variety of ambient dusts and is easily breathed (Thorne, 2000; Gereda et al., 2001). It is common for humans to be exposed to LPS on a regular basis (Baron, 1996).

Causes of endotoxin

Endotoxin is primarily caused by the release from bacterial and microbial breakdown. The presence of endotoxin in the environment is caused by the usage of antibiotics in hospitals and biological waste. Endotoxin is most dangerous when it is released from the bacterium, either in small amounts from rapidly developing cells or in higher concentrations by physical, chemical, or mechanical cell lysis (Khan et al., 1998). The main source of endotoxin in the environment is the natural breakdown of bacteria and their usage in the pharmaceutical business.

Sources of endotoxin

Natural and anthropogenic sources are the two main sources of endotoxin in the environment. Natural sources are windblown dust from soils, sea spray, or allergens from fungus and plants, while manmade sources include sewage treatment plants, hospital waste, combustion byproducts (energy generation); or a combination of the two. Farms with composting facilities - swine, poultry, and cattle; sea-spray; agriculture dust; animal dander, hair, feathers, urine and faeces, insects, mites, pollen grains, and other natural sources are primary causes of outdoor particle exposure. Sorting and composting garbage (sorting, shredding, turning, and screening waste); water used as a solvent in packing components, raw

materials, equipment, and medications; wastewater treatment plants, including pharmaceutical industries, are anthropogenic sources.

Endotoxin effects

Endotoxin affects human health, plants and animals. Endotoxin has long been recognized as a workplace hazard in a variety of industries, including swine, poultry, and dairy barns; grain and animal feed handling facilities; vegetable, hops, mushroom, and cotton processing; sawmills; metal machining; fiberglass production; and composting and waste management. Endotoxin has two different sorts of health impacts: biological effects on hosts infected with Gram-negative bacteria and health effects.

Biological effects on Gram-negative bacteria-infected hosts Immune reaction

The pathogens that cause meningococcal disease, including meningococcaemia, Waterhouse–Friderichsen syndrome, and meningitis, are mostly responsible for the dramatic clinical manifestations of infections with pathogenic Gram-negative bacteria, meningitides, and meningitis. Alkaline phosphatase inhibits intestinal inflammation (and "leaky gut") by dephosphorylating the lipid A part of LPS. LPS can stifle their ability to repopulate in a competitive environment. The prototypical endotoxin is LPS. Exogenous pyrogens include LPS (fever-inducing substance). Intestinal alkaline phosphatase dephosphorylation of LPS can lessen the severity of *Salmonella typhimurium* and *Clostridioides difficile* infection while restoring normal gut microbiota. These compounds are important to Gram-negative bacteria and they could be potential targets for novel antimicrobials. LPS sensitivity is substantially higher in humans than in other species like; mice. In humans, a dose of 1 g kg^{-1} causes shock, whereas mice can withstand doses up to a thousand times greater.

Variability's impact on immunological response

The innate immune system's toll-like receptors identify LPS and initiate an immunological response. Antigenic specificity is imparted by O-antigens (the outer carbohydrates), which are the most changeable section of the LPS molecule. Lipid A, on the other hand, is the most conserved component. These LPS may have antagonistic effects because of differences in lipid A composition.

Non-canonical LPS recognition pathways

LPS stimulates additional elements of the sensory system at increasing doses.

Endotoxin effects on human health

Endotoxin affects human health and causes several diseases like; endotoxemia, autoimmune illness, obesity, senescence of cells, etc.

Endotoxemia

Endotoxemia is the presence of endotoxins in the blood. Septic shock can result from a high amount of endotoxemia. Metabolic endotoxemia is characterized by a decreased concentration of endotoxin in the bloodstream. Obesity, nutrition, cardiovascular disease, and diabetes are all linked to endotoxemia, which can also be influenced by host genetics.

Septic shock can be caused by the unregulated activation of mammalian immune systems, which results in the creation of inflammatory mediators. Inflammatory mediators can cause capillary leak syndrome, blood vessel dilatation, and a reduction in heart function by damaging the endothelial layer of blood vessels. Disseminated intravascular coagulation (DIC) can result from high bacterial growth causing severe endothelium injury, resulting in the loss of function of internal organs such as the kidneys, adrenal glands, and lungs. Vascular damage can be visible on the skin. Endotoxin can also damage the limbs, with fatal results such as the development of gangrene, which may necessitate amputation. The adrenal insufficiency causes a loss of function. It can potentially result in a secondary hemorrhage. Waterhouse-Friderichsen syndrome can be fatal. Human fallopian tubes may also get damaged.

Autoimmune illness

Autoimmune illness is a condition in which the body's immune system attacks itself. Some molecules are thought to trigger autoimmune-based host reactions, such as multiple sclerosis flare-ups. *Helicobacter pylori*, and *Campylobacter jejuni* cause gastrointestinal disease in humans, and *Haemophilus ducreyi* causes chancroid.

Obesity

Endotoxin is associated to obesity. Endotoxin triggers an inflammatory response, which contributes to obesity and insulin resistance when germ-free animal models are injected. Purified endotoxin from *E. coli* can promote obesity and insulin resistance. Increased populations of endotoxin-producing bacteria

in the digestive tract are connected to specific obesity-related patient groups with higher endotoxin loads (Moreno-Navarrete et al., 2012; Lepper et al., 2007; Ruiz et al., 2007).

Senescence of cells

Endotoxin causes senescence of cell. LPS-induced inflammation can lead to cellular senescence.

Endotoxin effects on animals

Endotoxin affects animals also. Endotoxin causes physiological problems in animals as it is poison. Endotoxin causes disease in a variety of organisms. High concentration of endotoxin causes shock, diarrhea, depression, gut barrier difficulties, respiratory distress, and vasomotor irregularities.

Endotoxin effects on plants / vegetation

Endotoxin has a big impact on the flora as well. Endotoxin generated by gram-negative bacteria's outer membrane has a significant economic impact on plants such as cabbages, tomatoes, and grains. Plant breeding and genetically engineered plants cause poor immune responses.

Endotoxin causes injury to humans, animals, and plants, as well as financial loss. Endotoxin's effects are proportional to its concentration or level. Endotoxin testing is crucial.

Endotoxin detection techniques

Endotoxin can be measured using a variety of methods. The bacterial endotoxins test (BET) uses amoebocyte lysate from the horseshoe crab to identify or quantify endotoxins from Gram-negative bacteria (*Limulus polyphemus* or *Tachypleus tridentatus*). This test can be done in three ways:

Method A: The gel-clot approach is based on gel production. Endotoxin is detected or quantified using the gel-clot technique, which involves clotting of the lysate TS in the presence of endotoxin. The labelled sensitivity of the lysate TS is the minimal concentration of endotoxin required to cause the lysate to clot under standard conditions.

Method B: The turbidimetric technique is based on the turbidity that develops after an endogenous substrate is cleaved. The turbidimetric approach is a photometric assay that measures changes in the turbidity of the reactant. This approach can be classed as either an endpoint-turbidimetric assay or a kinetic-turbidimetric assay depending on the assay principle used. The quantitative relationship between the concentration of endotoxins and the turbidity (absorbance or transmission) of the reaction mixture at the end of an incubation time is the basis of the endpoint-turbidimetric assay. The kinetic-

turbidimetric assay is a method for determining the time (onset time) required to obtain a predetermined absorbance or transmission of the reaction mixture, as well as the rate at which turbidity develops. The test is performed at the lysate manufacturer's specified incubation temperature (typically 37±1 °C

Method C: The chromogenic technique is based on the development of color after cleavage of a synthetic peptide-chromogen complex. Chromogenic technique is an assay to measure the chromophore released from a suitable chromogenic peptide by the reaction of endotoxins with lysate. On the basis of the particular assay principle employed, this technique may be classified as either an endpoint-chromogenic assay or a kinetic-chromogenic assay. The endpoint- chromogenic assay is based on the quantitative relationship between the concentration of endotoxin and the release of chromospheres at the end of an incubation period. The kinetic- chromogenic assay is a method to measure either the time (onset time) needed to reach a predetermined absorbance of the reaction mixture, or the rate of color development. The test is carried out at the incubation temperature recommended by the lysate manufacturer (which is usually 37 ± 1 °C).

The Limulus amebocyte lysate (LAL) test for endotoxin quantification and the nuclear factor kappa B (NFB) activation assay were used by Mahapatra et al., (2018) to quantify inflammatory activity (Mahapatra et al., 2018). Several authors have looked into the link between endotoxin and inhalable dust.

The significance of the endotoxin research

Endotoxin is a biological component of particulate matter and can have negative health consequences. Bacteria are inevitably regular visitors to the human respiratory system. Human lungs are exposed to roughly 8000 liters of inhaled air each day and comprise the largest interaction between the human host and the external environment. Although exposure to microorganisms and their components is necessary for immune system development (hygiene hypothesis) but endotoxin from gram-negative bacteria can pose a health risk and be life-threatening. Air pollution is now the most serious environmental health threat on the planet. Particulate matter contains biological materials that contribute 5-40%, yet inhalation of air causes oxidative stress and airway inflammation. Fine particulate matter is a substrate for endotoxin and persists in the air. Bacterial endotoxin (or endotoxin)

is a toxin produced by bacteria. Gram-negative bacteria's cell walls contain bacterial endotoxin (or Lipopolysaccharide), which has a severe proinflammatory effect. As a result, it is critical to understand the presence of endotoxin in our environment in order to determine their role in PM-related inflammation and to take control measurements for human safety.

2. REVIEW OF LITERATURE

The study of endotoxin has attracted the scientific community's interest. However, endotoxin research is still in its early stages, and there are not enough datasets. Because of the significant impact of endotoxin and the biological component of suspended particulate matter on human health, researchers are interested in investigating suspended endotoxin, which can trigger inflammatory reactions when inhaled or deposited. Respiratory problems, allergies, cancer, and infectious disorders are all thought to be caused by endotoxin in the environment. This overview of the literature summarizes endotoxin concentration in the air and their consequences on human health and the environment

International

Endotoxin is a biological component of particulate matter (PM) and when mixed with other contaminants causes negative health effects and potentially aggravate ailments when inhaled (Guan et al., 2014). The results of research into the link between air pollution and health have been published. Endotoxin levels have been detected in a variety of locations across the world. In terms of area, the geometric mean of PM2.5 concentration and fraction of endotoxin was 0.65 EU m^{-3} and 10.25 EU mg^{-1} (Guan et al., 2014).

In residential, school, and business settings, mean endotoxin loads in settled floor dust ranged between 660 and 107,000 EU m^{-2}, 2180 and 48,000 EU m^{-2}, and 2700 and12,890 EU m^{-2} respectfully (Salonen et al., 2016). In households, mean endotoxin concentrations ranged from 0.04 to 1610 EU m^{-3}, while in businesses, they ranged from 0.07 to 9.30 EU m^{-3}.

The amount of endotoxin in interior dust is affected by the age of the house, cleaning, flooring materials, the number of residents, the presence of dogs or cats, and the relative humidity (Salonen et al., 2016). Mahapatra et al., (2018) reported PM10 concentrations between 136 to 189 µg m^{-3} and endotoxin concentrations varied between 0.29 to 0.53 EU m^{-3} at several places in Chitwan, Nepal. The

quantities of endotoxin (0.132 EU m^{-3}), PM10 (51.9 µg m^{-3}), PM2.5 (22.6 µg m^{-3}), and O3 (0.018 ppm) were calculated by Hwang et al., 2019.

Endotoxin levels were observed to vary substantially depending on the season, with high levels being typical in the spring and winter (Guan et al., 2014). In South Korea, endotoxin levels are higher in the autumn and winter than they are in the summer (Hwang et al., 2019). Airborne endotoxin is associated with other components of environmental and climate factors. Temperature and relative humidity, as well as sulphur dioxide and nitrogen oxide levels, were found to be significantly related to airborne endotoxin levels ($p < 0.05$) (Guan et al., 2014). The concentration of endotoxin was found to be highly correlated with ambient temperature ($p<0.05$) (Degobbi et al., 2010).

The majority of Gram-negative bacteria and cyanobacteria produce endotoxin, which is a Lipopolysaccharide (Duquenne et al., 2013). Gram-negative bacteria numbers in pure bacterial cultures or associated samples are frequently connected with LAL endotoxin assays (Hurley and Tosolini et al, 1992; Anderson et al., 2002). The amount of endotoxin present in Gram- negative bacterial cells is poorly understood. Bacterial cells contain between 10 and 40 fg of LPSs (Hurley, 1995; Anderson et al., 2002). In several investigations, airborne endotoxin levels (measured using the approach) are well connected with culturable Gram-negative bacteria concentrations (Alwis et al., 1999; Gora et al., 2009) or airborne microorganisms evaluated using other methods (Laitinen et al., 1992; Alwis et al., 1999; Reiman and Uitti, 2000; Su et al., 2001). There have been several studies published on the association between fungus and endotoxin. Particulate matter, particularly PM2.5, has been associated with increased morbidity and death from respiratory illnesses (Degobbi et al., 2010). Fungi and endotoxin account for a significant percentage of PM2.5, with average concentrations of 772.23 spores mg^{-1} and 5.52 EU mg^{-1} of PM2.5, respectively.

The existence of ambient endotoxin in PM10 and its link to inflammatory activity, air pollutants, and meteorology were examined by Mahapatra et al., (2018). Endotoxin levels were found to be positively related to pollutant levels (Mahapatra et al., 2018) implying a common source. There were also associations between different endotoxin particle size fractions and dust particle size fractions (Madsen and Nielsen, 2010). Guan et al., (2014) collected the first continuous time series of airborne endotoxin concentrations in Beijing and found plausible links to atmospheric variables. Endotoxin

concentrations were found to have positive relationships with Na^+, K^+, Mg^{2+}, and F^-, but negative correlations with P, Co, Zn, As, and Tl. Endotoxin concentrations were found to be positively related to reactive oxygen species (ROS), but not to dithiothreitol (DTT) of particulate matter (Guan et al., 2014). Hwang et al., (2019) measured airborne endotoxin levels in South Korea and compared them to ambient PM10, PM2.5, and O3 levels, as well as environmental factors that influence these values. Hwang et al., (2019) obtained a negative correlation between endotoxin levels and O3 levels ($r = -0.491$), and endotoxin levels and temperature ($r = -0.302$) were found to have negative associations concluded that more research with larger sample size is needed to establish the causal processes.

Using a biomarker technique, source identification research for airborne bacteria and fungus was also carried out. Lee et al., (2006) reported ergosterol as biomarkers to identify airborne endotoxin from Gram-negative bacteria and fungal biomass in the atmosphere. Principal component analysis (PCA) and backward air mass trajectory analysis were used identify the sources. The bacterial endotoxin was detected in the same factor group as Ca^{2+} in coarse particles (PM2.5–1.0) and accounts for about 20% of the overall variance in the PCA, implying that the airborne bacterial assemblage has a crustal origin. Endotoxin was found to be inversely related to inflammatory activity as a result of a time-limited forest fire incident. Biological components (bioaerosols) have been linked to air quality degradation and human health effects in a variety of settings (Lacey and Dutkiewicz, 1994), including agriculture (Heederik et al., 1991; Kullman et al., 1998), metal machining (Thorne and DeKoster, 1996), and residential environments (Michel et al., 1991; Michel et al., 1996). PM2.5 has been reported to be associated with the exacerbation of asthma, bronchitis, chronic obstructive pulmonary disease (COPD), and lung cancer (Bonner, 2007).

Significant correlations have been discovered in a variety of industries, including waste composting (Schlosser et al., 2009; Sykes et al., 2009), agriculture, agro-industry, animal husbandry (Roy and Thorne, 2003; Madsen et al., 2009; Thorne et al., 2010), wood industry (Spankie and Cherrie, 2012). While moderate correlations were found in the cotton sector (Astrakianakis et al., 2006; Paudyal et al., 2011) and farms (Traversi et al., 2011), whereas weak correlations were found in sawmills (Douwes et al., 2000; Astrakianakis et al., 2006; Paudyal et al., 2011).

Endotoxin exposure can cause inflammatory reactions in the blood and lungs, resulting in respiratory symptoms as shivering chills, fever, and severe asthma (Michel et al., 1992, 1996, Rennie et al., 2008; Park et al., 2000, 2001). Ryan et al., (2009) looked into traffic-related particles and endotoxin exposure in children, as well as persistent wheezing. Endotoxin exposure was found to have a synergistic effect with traffic exposure on chronic wheeze, which has been linked to poor respiratory outcomes (Mahapatra et al., 2018). Household dust containing endotoxin may play a role in the severity of bronchial allergies in residential settings (Rylander et al., 1989; Michel et al., 1996; Gehring et al., 2002). Indoor air filtration was explored as a potential cause of elevated airborne endotoxin levels, and stand-alone portable air purifiers (APS) were discovered to be an increasingly popular technique of minimizing individual inhalation exposure (Niu et al., 2020). Mendy et al., (2019) looked at the synergistic relationship between house endotoxin exposure and ambient air pollution and asthma outcomes. In children, co- exposure to elevated endotoxin and NO_2 was demonstrated to have a synergistic effect. Endotoxin can also cause obesity.

National Status

Bio-aerosols (short for biological aerosols) are a type of particle produced by terrestrial and marine organisms and released into the atmosphere. Fungus, bacteria, viruses, pollen, endotoxin, and other living and non-living components are found as important component of bioaerosol. The outer membrane of bacteria produces endotoxin. The majority of the research is focused on the indoor environment. In the living room, kitchen, bedroom, toilet, and office, the amount of airborne bacteria and fungus was assessed. Aspergillus and Penicillium were the most prevalent fungi detected in the residence, while Staphylococcus and Micrococcus were the most common bacteria. The average indoor endotoxin concentration ranged from 6 to 39 EU m^{-3} (Balasubramanian et al., 2011). The amount of ventilation and the types of human activities carried out in the indoor environment were found to be important factors in determining the level of these airborne biological contaminants. Seasonal effects of a sewage treatment facility on endotoxin levels at various locations during five seasons (aeration tank, sludge bed, grit chamber, and final settlement tank) have been reported. The endotoxin concentration was found to be highest at the aeration tank during the summer season and lowest at the FST during the winter season in the size range of (PM10-2.5), both of which are

significant. The concentration of endotoxin was found to be considerably different ($p < 0.05$) in the summer and winter seasons across all sections of the country, but not statistically different ($p > 0.05$) in the monsoon and autumn seasons (Maharia et al., 2020). Airborne endotoxin and β-D-glucan in PM1 have been studied in agricultural and domestic settings. The geometric mean of airborne total endotoxin concentrations on farms and in households was 1.20 ×104 (7.57) EU m^{-3} and 2.67 (3.82) EU m^{-3}, respectively (Singh et al., 2011). The relative amounts of PM1 endotoxin in dwelling samples (7.9%) were significantly higher ($p < 0.001$) than in agricultural samples (0.3 percent). Residences contain higher levels of PM1 endotoxin and β -D-glucan than farms. These findings emphasize the importance of doing size-selective air sampling in homes to assess microbial exposure.

In rural India, biomass fuel-burning houses (Gangamma et al., 2020) are a significant source of negative health effects. Particulate matter containing levoglucosan is suggested to be a biomarker for biomass combustion. Carbohydrate and levoglucosan concentrations in the air range from 3.5 to 119.7 µg m^{-3} and 1.9 to 77.9 µg m^{-3}, respectively. LPG-using households had considerably lower amounts of airborne carbohydrate and levoglucosan than biomass-using households. Carbohydrate intake is positively related to particulate matter mass concentration. Protein levels were found to be positively associated with particulate matter mass and carbohydrate levels in homes that used biomass fuel. The median levels of airborne bacteria in LPG and biomass-burning households were 215 CFU m^{-3} and 323 CFU m^{-3}, respectively. Gangamma et al., 2021 demonstrated the levels of bio components connected to particulate matter in Delhi city. Particulate matter (PM) is made up of 16 to 25% bio-components (Fröhlich- Nowoisky et al., 2016), and it has the potential to harm humans' health and the ecosystem. Total protein levels in the city ranged from 13.4 to 55.5 g m^{-3}, while endotoxin values in PM samples ranged from 0.75 to 22 EU m^{-3}. Levoglucosan concentrations ranged from 1.5 to 6.7 µg m^{-3}. Protein, endotoxin, and levoglucosan concentrations were shown to be correlated with PM mass concentrations.

Carbohydrates can be used as a tracer for specific sources of airborne particulate matter (PM). Levoglucosan is used as a biomass combustion marker. The amounts of total carbohydrates and Levoglucosan in the samples were also measured in India by Gangamma et al., (2020). Airborne endotoxin concentrations varied from 0.03 to 1.1 EU m^{-3}, with an average of 0.3 EU m^{-3}. Gangamma

et al., (2020) has reported significantly higher carbohydrate concentrations at Chandigarh than other Indian cities. Gangamma et al., (2020) provided the data on the concentration and characterization of airborne biological particles in six Mumbai wastewater treatment plants and found 49 % of the sample for airborne endotoxin and bacteria exceed the occupational exposure limit.

In an in vitro experiment, endotoxin was shown to be the single most important component of particulate matter responsible for triggering the pro-inflammatory signal (tumor necrosis factor- a). Gangamma et al., (2014) studied the role of bacterial endotoxin in the ex vivo particulate matter induced proinflammatory response by identifying airborne bacteria in the Mumbai metropolitan environment. Pathogen sensing employing particular receptors was studied by Mishra et al., (2020). The highest bacterial aerosol concentrations associated with PM10 size fraction were found in urban settings, while the highest fungal concentration was found in rural households (Roy et al., 2020). Mamta et al., (2016) found bacterial and fungal concentrations in the respirable suspended particulate matter at the sub-urban, whereas Raghav et al., (2020) found biological components in PM10 and PM2.5 at an urban site in the Agra region in the Indo- Gangetic basin. The impact of climate change on the number of viable bioaerosols has been carefully investigated (Mamta et al., 2016). Bioaerosols and particle concentrations on the Indo-Gangetic Plain, as well as the impact of biomass burning emissions on high-level endotoxin exposure, were studied by Rajput et al., (2017).

Bioaerosol, significant constituents of particulate matter contain a significant amount of endotoxin. Bioaerosols are a type of particle produced by terrestrial and marine ecosystems and released into the atmosphere. Bioaerosols include fungi, pollen, bacteria, and viruses, as well as endotoxin, mycotoxins, and allergens, which are all well-known components of indoor and outdoor air processes. Microbiological diseases, endotoxin, and allergens to which humans are allergic are all disseminated by them. Exposure to endotoxin has been linked to a number of health issues. Due to time-consuming testing processes, endotoxin, on the other hand, has gotten the least attention. The lack of a worldwide standard technique for collecting and evaluating airborne endotoxin is largely responsible for the lack of an occupational exposure limit. The LAL test is the most often used analytical procedure. However, little research has been done in this sector in India so far. The current COVID-19 epidemic has

underlined the crucial need for bioaerosol research. Research relevant to the present pandemic is critical, as more pandemics may arise if ambient air quality continues to deteriorate.

3. OBJECTIVE

Endotoxin is a pyrogenic toxic molecule of biological origin. When gram-negative bacteria die and their cell walls breach, endotoxin is released into the environment. Pneumonia, influenza, measles, asthma, allergies, and obesity are all caused by exposure to endotoxin. Endotoxin levels differ by location, year to year and season to season due to variation in regional climatic condition as well as sources and distributions. Endotoxin, on the other hand, in India has gotten the least attention so far. Because of the current pandemic crisis, risk of more pandemics in the coming years, and the worsening of ambient air quality day by day, it is important to study the concentration and effect of endotoxin. The study on endotoxin concentrations in suspended particulate matter of different sizes, source characterization, effects of environmental and climatic factors on concentration of endotoxin as well as health risk assessment are quite important. With this in view the objectives of the present investigation are:

- to determine the concentrations of PM10, PM2.5, and PM1 particles in the ambient air in Agra over the IGB region,
- to characterize the annual and seasonal variation in the concentration of endotoxin,
- to determine the association of endotoxin with other biological components of aerosol,
- to determine the climatic effects on endotoxin concentrations,
- to assess the health risk of endotoxin and biological components of aerosols, and
- to provide datasets for regional health model

4. MATERIALS AND PROCEDURES

Site selection

The intended research would take place in Agra over the semiarid region of the Indo-Gangetic basin. Agra is 200 kilometers southeast of Delhi, in north-central India, on the Indo-Gangetic basin (27.1767° N, 78.0081° E). Agra is a well-known tourist destination as it is home to "the Taj Mahal" and other cultural treasures. Two-thirds of its periphery is covered by the Thar Desert of Rajasthan, which has a sandy-loamy soil type with a lot of salinities. Summer (March-June), monsoon (July-

September), post-monsoon (October-November), and winter (December-February) are the four seasons of Agra. The major wind directions in Agra are north and northwest, with wind speeds ranging from 1-2 m s^{-1}.

Sampling location

In Agra, sampling will be carried out on the roof of the Technical College of Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra. Dayalbagh is a suburban site of Agra and is 10 kilometers away from the city's industrial zone, where agricultural activities have resulted in vegetation dominating. The sampling location is on the side of a road with mixed vehicular activity. A National Highway (NH-2) is 2 kilometers south and has a high volume of traffic. Aside from local sources, the refinery in Mathura and glass industries in Firozabad is 40 kilometers west and east of Agra, respectively.

Sampling - Aerosol particle sampling and sample preparation

The aerosol particles will be collected in a variety of aerodynamic diameters. PM_{10} atmospheric particulate matter with an aerodynamic diameter of 10 μm, $PM_{2.5}$ with an aerodynamic diameter of 2.5 μm, and PM_1 with an aerodynamic diameter of 1 μm will be collected for 24 hours using sterile Glass fiber/PTFE/Quartz filter paper as collecting surface by Envirotech / Polltech dust samplers at a flow rate of 16.67 L min^{-1}. The sterile filter will be weighed and stored in a desiccator before and after the sampling. The sampling will be done once a week.

The gravimetric analysis will be used to determine the mass concentrations of PM_{10}, $PM_{2.5}$, and PM_1 in the ambient air. The difference in mass of the filter before and after sampling to total air volume (multiplication of flow rate and sample length) will be used to compute the ambient mass concentration of particles, which will be expressed in μg m^{-3}.

Concentration (μg m^{-3}) = Mass of filter paper (after sampling-before sampling) / Total air volume (m^3)

A field blank will be collected in the same way as a sample. The PM_{10}, $PM_{2.5}$, and PM_1 filters will be split into two portions. One part of each filter will be cut into small pieces and extracted for two hours on an electrical shaker in sterile E-pure (pyrogen-free water), followed by 30 minutes on the ultrasonic bath. The endotoxin and biological components of particulate matter will be analyzed in the supernatant of a water extract.

Dry deposition sampling on surrogate surfaces

A polypropylene tray will be used to collect dry deposition samples. The polypropylene tray will be cleaned with sterile deionized water, dried using an IR (infrared) lamp, and then exposed for 96 hours on the roof of the building. The dry deposition sample will be collected by the surface washing method. The particles will be filtered via sterile Whatman filter paper after they have been collected. The filtrate will be used for the endotoxin analyses.

Field blanks will be collected in the same way as the dry deposition is done: the tray will be exposed for one minute.

Soil sampling and preparation

Soil is a significant pollutant source and sinks. The soil in this area could be a major source of endotoxin. During the study, soil samples will be collected and tested for endotoxin. Topsoil will be collected from a variety of areas around the sampling sites, air-dried, and sieved through a 60-mesh sieve. 0.1 gram of sieved soil in 10 ml ultrapure water (pyrogen-free water) will be extracted in an electrical shaker for around 2 hours, followed by 30 minutes in an ultrasonic bath. Extracted soil samples will be filtered and endotoxin analysis will be performed.

Analysis - Endotoxin Analysis

Endotoxin can be detected using a variety of approaches. The LAL (Limulus Amebocyte Lysate) assay has the advantage of detecting physiologically active endotoxin and allowing for large-scale analysis.

All samples shall be collected in pyrogen-free polypropylene bottles or glass bottles. Precautions will be taken to avoid breathing on, touching, or otherwise exposing the sample containers to human contamination, including the use of gloves when connecting or disconnecting the cassette and the pump. Following sampling, the inlet and outlet of the cassette will be covered with a protective covering back (cap), and the cassette will be put in its original packaging and taped shut. The samples will be maintained at 4.2°C till analysis is completed.

Endotoxin in dry deposition and soil samples will be determined using a contamination-free LAL assay (Associations of Cape Cod, Inc., USA). However, because the LAL assay does not detect membrane-bound LPS, it may underestimate the quantity of endotoxin present in the sample that could

be liberated by other methods, either in the environment or once breathed into the lungs (e.g., dissociation from the cell wall by alveolar phagocytic cells). The rate of activation in the modified lysate is related to the amount of endotoxin in the sample (Sarkar et al., 2015; Thorne et al., 2010). The entire endotoxin extraction procedure will take place at room temperature.

One milliliter of water extract from the aerosol sample will be poured into a cryovial. Five-fold serial dilutions will be used to test the filter extracts. The Control Standard Endotoxin will be tested at two-fold dilutions to create a 12-point standard curve ranging from 0.0244 EU mL to 50.0 EU mL. The samples and field blanks will be evaluated in 96 well microplates (Corning Inc, Corning, NY), with the rate change of absorbance measured every 30 seconds at 405 nm for 90 minutes using a micro plate reader (Maharia et al., 2020).

$$\text{Concentration of endotoxin (EU m} - 3) =$$

$$\frac{(Concentrationinsample \times Dilutionfactor \times Extractionvolume)}{\text{Air Volume sampled in L}} \times \frac{1000L}{m^3}$$

Endotoxin in dry deposition and soil samples will be analyzed in the same way that aerosol samples. The endotoxin dry deposition flux (EU m^{-2} day^{-1}) will be calculated by dividing the total endotoxin unit by the area and number of sampling days. The endotoxin in the soil sample will be evaluated in the same way that the endotoxin in aerosol samples is, and the results will be provided as EU per gram of soil.

Endotoxin-free consumables and glassware will be utilized. Negative controls will be used throughout the analytical method to confirm that contamination is not present.

Biological components of aerosol

The biological components (bacteria and fungi) of aerosol particles will be estimated. One milliliter of aqueous extract of filter will be used to characterize and determine the concentration of biological components in aerosols, the presence of bacteria, and fungi will be determined using the culture technique.

Identification and purification of fungi and bacteria

SDA (Sabouraud Dextrose Agar) media will be used to examine the fungi in the aerosol sample. The Sabouraud Dextrose Agar (SDA) medium will be made using 10 g peptone, 40 g dextrose, and 20 g

peptone in 1000 ml sterile E-pure water. The pH will be kept constant at 5.6. A spirit lamp will be used to disseminate 1 milliliter of aqueous particulate matter extract across Petri plates containing 20-30 ml SDA medium in a laminar flow for 3-5 days. Petri plates will be incubated at 27°C in a BOD incubator. The number of colonies generated will be counted. The fungus spores will be identified using an oil immersion light microscope (M/s. Motic) at a magnification of 100x. On a slide containing 4 percent NaCl, a little portion of the fungal colony will be inserted. A drop of cotton blue stain will be applied, the area will be covered with a cover slip, and the area will be left to dry for around 1-2 minutes. The fungus will be recognized and identified based on the colour, shape, kind, and structure of the spore (Campbell and Johnson et al., 2013). With the use of contemporary literature and laboratory guides (Manual of Chrysoporium and Allied Taxa, and a colour Atlas of Pathogenic Fungi). Sub-culturing will be used to purify fungi until a pure form is obtained.NAM will be used to cultivate bacteria (Nutrient Agar Media). 1 g beef extract, 2 g yeast extract, 5 g peptone, and 5 g NaCl will be added to 1000 ml sterile E-pure water to make Nutrient Agra Media. A spirit lamp will be used to disseminate 1 ml of aqueous extract of particulate matter sample across a Petri plate containing 20-30 ml of NAM in the laminar flow. The plate will be incubated at 35°C for 24 hours before being counted for spores. A light microscope (Motic Microscope) with a magnification of 100x will be used to identify the bacterial spores morphologically. Staining processes will be used to distinguish between Gram-positive and Gram-negative microorganisms. By streaking the bacterial colony over agar plates multiple times, a pure culture of bacteria will be obtained. On the basis of physical traits, the colonies will be segregated.

The amount of fungus and bacterial spores in the air will be estimated in CFU (colony forming unit) per cubic meter (m^3).

Number of colonies x dilution factor / total air volume (m^3) = concentration (CFU m^{-3})

SEM Analysis

A scanning electron microscope (SEM) with an electron dispersive X-ray will be used to analyze the form and size of bacterial and fungal components of aerosol particles, as well as associated chemical contents. The colony will be mounted on aluminum stubs, coated, and analyzed with magnifications of 500x or 3000x.

Risk assessment for health

Endotoxin can be found in the air as a solid or in combination with other biological and non-biological components, and it can cause health problems (Duquenne et al., 2013). The health risk assessment will be carried out by calculating the amount of endotoxin inhaled and creating a link between endotoxin concentration and hospital admission based on these findings.

Endotoxin inhalation health risk evaluation

The particle lung deposition model will be used to estimate endotoxin deposition in the human respiratory system. The amount of endotoxin deposition in the human airway will be measured using the ICRP (International Commission on Radiological Protection) model. The head airway (HA), the tracheobronchial area (TB), and the alveolar (A) region will be divided into three sections. Each size range's geometric mean diameter (D_P) will be established, and the deposition efficiency of particles in each size range will be calculated.

$$D_{HA} = I(D_P)\left(\frac{1}{1 + 934D_P^{1.183}} + \frac{1}{1 + 2.52D_P^{-1.885}}\right)$$

$$I(D_P) = 1 - 0.5\left(1 - \frac{1}{1 + 0.0007D_P^{2.8}}\right)$$

$$(D_P) = \frac{0.00352}{D_P}[exp(-0.416(\ln D_P + 2.84)^2) + 19.11\exp(-0.482\ (\ln D_P - 1.362)^2)]$$

$$D_A(D_P) = \frac{0.0155}{D_P}[exp(-0.416(\ln D_P + 2.84)^2) + 19.11\exp(-0.482\ (\ln D_P - 1.362)^2)]$$

Endotoxin inhalation health risks will be compared based on the number of endotoxin deposited in each location. The number of endotoxin deposited in each area will be used to compare the health consequences of endotoxin inhalation.

Establishing a link between hospitalizations and endotoxin concentrations

The link between hospital admission and endotoxin concentration will be established. The endotoxin components of aerosols have been reported to have negative effects on human health. A relationship between hospital admission and endotoxin concentration will be established to assess the health impact of endotoxin on human health. The information on human hospital admissions will be acquired from the OPD (Out Patient Department) of Agra's most prestigious hospitals (SN Medical College

etc.). The data will be collated and categorized into categories like sickness, gender, age, and geographic area. The correlation and regression on the secondary datasets obtained, with endotoxin will be done, and a relationship between endotoxin concentration and disease will be established.

Statistics analysis

The entire data set will be statistically analyzed, including mean, standard deviation, ANOVA test, correlation analysis, regression analysis, and factor analysis, using the SPSS +11.0 software packages. The ANOVA test will be used to determine the significance of the variance in the mean; correlation and regression analysis will be used to determine dependencies of variable dependencies; and factor analysis will be used to identify potential sources.

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